REMARKS

Reconsideration of this application is requested.

At the outset, the undersigned wishes to thank the Examiner (Mr. Kushan) and his supervisor (Ms. Moskowitz) for kindly agreeing to conducting a personal interview on this application. The interview was conducted on August 16, 1988, and the courtesies extended by the Examiner and his supervisor were most appreciated.

The purpose of the interview was to discuss the objections and rejections in the outstanding official action. The points discussed are dealt with item by item below, with reference to the numbered paragraphs of the official action.

15-18. The provisional election of claims 1-4 is hereby affirmed. Although these claims are now cancelled, claims 17 and 18 are of the same category and claims 19 and 20 recite the same features as 17 and 18. A divisional application has been filed in respect of cancelled claims 5-16.

21. Applicants accept the principle of the Examiner's point, that they have to distinguish in some way over purified human factor IX obtained from blood plasma in order to overcome a 35 USC 102 rejection. There are two ways in which the recombinant factor IX differs from human factor IX. One is that recombinant factor IX is free from all plasma contaminants. It is notorious that high molecular weight proteins are <u>extremely</u> difficult to purify to an extent of removing <u>all</u> contaminating

proteins. For example, obtaining what appears to be a single band on a gel is certainly non-evidential of complete purity. The references cited on this point are summarized as follows:

Osterud et al describes a crude purification method based on chromatography on a heparin column.

Schwinn et al describes a procedure in which a crude concentrate from plasma is heated with a calcium salt, glycine and sucrose to remove hepatitis viruses. No attempt is made to remove other plasma constituents.

Anderson et al describes another crude purification based on chromatography on a heparin column. The 52 times purification of Example II sounds impressive, but is in fact very low (compared Suomela et al's 9,500 times).

Suomela et al (see the full paper, which is enclosed as applicant's reference AAA), of the four references cited, is the only serious attempt at high purification of factor IX. However, the product is obviously not free of all plasma constituents. Thus, gel 4 in Figure 3 on page 150 is not completely free of high molecular weight material towards the top of the band. In isoelectric focussing (Fig. 4), the peak is unsymmetrical (Fig. 4A) or split (Fig. 4B), indicating the presence of impurity.

Moreover, there is apparently some polymorphism or impurity affecting the terminal amino acid, which was indicated to be only 95% tyrosine by N-terminal analysis, see page 149, right-hand column top. As discussed below in connection with the McGraw et al reference (AAE), it is not a polymorphism and so it

must be due to an impurity.

Since even the highly purified product of Suomela et al is contaminated by its source, it is clear that the far less purified products of the other three references must be even more contaminated. Thus, it is believed that the recitation in claim 17 that the product is free of plasma constituents is adequate to overcome the 35 USC 102 rejection.

With reference to the requirement of the claims that the product be free of poxviruses proteins, this has nothing whatever to do with distinguishing over naturally extracted factor IX. It is simply to make clear that applicants are not interested in factor IX protein generated in tissue cells which have been infected by a vaccinia virus or like vector carrying the factor IX precursor gene. Vaccinia virus is still considered a dangerous pathogen. As pointed out in the introduction of the present specification, the French company Transgene claim to have made factor IX protein (of low activity) in this way.

It is also to be noted (as discussed during the interview) that there is a polymorphism in plasma human factor IX, by which amino acid 148 is threonine in some individuals but alanine in others, see R.A. McGraw et al, Proc. Natl Acad. Sci. USA 82, 2847-2851 (1985), now cited as reference AAE. This means that when human plasma is pooled to make a concentrate containing factor IX, and the factor IX is purified, the purified product is not a single protein. Further, McGraw et al indicate at page 2849, left-hand column, the paragraph beginning "Although

comparison..." two thirds of the way down, that this is the only polymorphism in the coding part of the genome (that which becomes translated into factor IX precursor and thereafter modified to form factor IX protein). This means that the lack of homogeneity in the very terminal amino acid sequenced by Suomela et al cannot be accounted for by a polymorphism but must be due to an impurity.

In order to yet further distinguish the claimed material from plasma human factor IX, the new claims presented with this response specify that the protein is derived from a single human individual. Thus, the claimed protein, in addition to having the features (1) through (3) as recited in new claim 17, also is polymorphism-free (i.e. monomorphic). Support for this amendment appears in the application as originally filed, for example at page 10 beginning at line 34 where it is indicated that the starting factor IX is the cDNA clone cVI described by Anson et al (reference AR). According to Anson et al, at page 1059 "Material and Methods", clone cVI came from "Library I". Library I is of cDNA prepared from mRNA extracted from "human liver" (left-hand column, 4th line between the italicized sub-heading). While there is no specific reference to "a human liver" in Anson et al, such language does appear in reference AL (page 24 line 4), which is the Assignee's European patent directed to the cloned gene. Both of the references AL and AR can be rationalized in that, in both instances, the cDNA was fractionated on a Sephacryl S 400 column and the first 70% of

the peak extracted with 1:4 butanol-chloroform (see page 25 lines 11 through 16 of AL and page 1059 left hand column of AR, lines 8 through 12 under the italicized sub-heading).

From the above comments, it is believed clear that the DNA coding for the factor IX protein as claimed in the present application is derived from a single individual human. In light of this, and in light of the other features recited in new claims 17 and 19, it is believed that the Examiner's anticipation rejections have clearly been obviated. Withdrawal of those rejections is therefore respectfully requested.

Turning to the outstanding obviousness rejections, it is believed, at the outset, that the claimed factor IX material would not have been obvious to a person of ordinary skill in the art in view of the teachings of the references relied on by the Examiner. As noted earlier, the only serious attempt to obtain highly purified factor IX is that reported by Suomela et al, and even there, impurities remain in the product. Furthermore, the Suomela et al purification is obviously impractical, so one would not conceive of taking their purification any further. Accordingly, it is believed that the 35 USC 103 rejection on that ground alone is not sustainable.

As further evidence of non-obviousness of the claimed invention, the Examiner's attention is directed to the three attached executed declarations, one by Professor Brownlee, who is one of the inventors, and the other two by independent experts, Dr. Tuddenham and Dr. Gitschier. The Examiner has

already seen copies of the attached declarations by way of a telefax transmitted to the Examiner on December 9 and December 12, 1988. That telefax transmission also included a proposed amended independent claim. The telefaxed materials were discussed during a telephone discussion with the Examiner on December 13, 1988, and the Examiner's helpful comments were most appreciated.

Referring specifically to the attached executed declarations, all three declarants are highly qualified in various ways. Professor Brownlee is a Fellow of the Royal Society and holds a prestigious chair at Oxford University. Dr. Tuddenham is a distinguished haematologist with a special knowledge of blood clotting and has been active in factor IX specifically in connection with monoclonal antibodies. Dr. Gitschier has worked for Genentech Inc. in the factor VIII recombinant DNA field.

Taking first Professor Brownlee's declaration, he indicates that he had poor expectations of success in research which ultimately led to this invention. The reason for these poor expectations lay in (1) the nature of the modifications which the factor IX precursor must undergo after translation and (2) the fact that tissue cells rather than live animals had to be used for the research. Professor Brownlee indicates that he was particularly doubtful about achieving the beta-hydroxylation (in any kind of cells) and about whether gamma-carboxylation would be achieved in tissue cells. A particularly telling point made

by Professor Brownlee is that although a cell line H4-11-E-c3 known to secrete prothrombin was chosen, it did not necessarily follow (1) that this cell line would be capable of the gamma-carboxylation required in the modification of factor IX precursor or (2) that it could carry out beta-hydroxylation. Although it was known that prothrombin requires gamma-carboxylation by a carboxylase dependent on vitamin-K, quantitative information was lacking. More importantly, the beta-hydroxylation believed required to modify the factor IX precursor is not a reaction which was believed required to modify prothrombin. In light of Professor Brownlee's evidence, the clear conclusion is that one skilled in the art would not have expected success in the attempt to produce fully biologically active factor IX protein.

In the copy of Professor Brownlee's declaration telefaxed to the Examiner, there were a number of manuscript interlineations of combinations of capital letters. As explained to the Examiner during the telephone discussion on December 13, 1988, those insertions refer to published literature references which support the statements made by Professor Brownlee. For completeness, a copy of the marked-up Brownlee declaration telefaxed to the Examiner is attached, together with copies of the published literature references and a completed Form PTO 1449. It is requested that the copy of the Brownlee declaration and each of the attached references be specifically made of record in the present application.

Dr. Tuddenham refers to the complications of the post-translational modifications. He mentions that he regarded the research leading to this invention as "highly speculative". Dr. Gitschier believes that success was by no means assured.

21A. The issue of entitlement to priority was discussed at the interview. It was pointed out that although the specific activity is not defined express verbis, it is arrived at by a fraction having the same denominator and numerator for which the data are given. See also the priority document, page 14 lines 19-23, which effectively divides 7 by 6.8 to give 100% = "fully active" protein. The term "fully active" is also present at lines 21-22. After reviewing the priority application the Examiner indicated that he would accord priority to the claims. Consequently, this rejection is believed overcome.

22. This paragraph contains a prior art rejection based on the purification of bovine factor IX, shown by Fujikawa et al. Bovine factor IX differs significantly from human factor IX. This can most easily be seen by comparing two published "sequence" papers, namely:

AAC Kurachi & Davie. Proc. Natl. Acad. Sci. USA 79, 6461-6464 (1982).

AAD Katayama et al, Proc. Natl. Acad. Sci. USA 76, 4990-4994 (1979).

Note the different compositions: Kurachi & Davie page 6462, RH column; Katayama et al, page 4991, middle.

Bovine Asp_{18} Asn_{23} Thr_{26} Ser_{33} Glu_{28} Gln_{12} Gla_{12}

Human Asp_{18} Asn_{28} Thr_{28} Ser_{23} Glu_{28} Gln_{13} Gla_{12}

Bovine Pro_{13} Gly_{34} Ala_{21} Val_{28} Met_{3} Ile_{23} Leu_{19}

Human Pro_{14} Gly_{35} Ala_{21} Val_{35} Met_{3} Ile_{21} Leu_{21}

Bovine Tyr_{18} Phe_{18} Lys_{30} His_{9} Arg_{18} Cys_{22} Trp_{8}

Human Tyr_{15} Phe_{20} Lys_{27} His_{9} Arg_{16} Cys_{22} Trp_{7}

The main compositional difference is that bovine has ten more serines, but seven less valines and five less asparagines than human. Of course, this crude comparison takes no account of sequence differences.

Since the Fujikawa et al reference relates to a different protein and does not describe a particularly high degree of purification, it is submitted that it is less relevant art than Suomela et al and that the rejection should therefore be withdrawn for the same reasons.

In light of the above, withdrawal of the outstanding anticipation and obviousness rejections is believed to be in order. Such action is requested.

23 & 24. In this paragraph, the Examiner has asked for a deposit of the hybridoma which secretes a monoclonal antibody to factor IX, on the ground that claims 1 to 4 allegedly recite a characteristic, viz specific activity, which is dependent upon a

monoclonal antibody. A deposit is not provided since a specific monoclonal antibody is not required. Any kind of monoclonal antibody to factor IX will do. The production of a hybridoma secreting monoclonal antibody to factor IX was a relatively simple matter, using the well known techniques. See, for example, A. Yoshioka et al, British Journal of Haematology 59, 265-275 (1985) at page 269 under "Results". (This paper was published on 14th February 1985 and is cited as reference AAB). The products of an ordinary mouse spleen cell/mouse myeloma fusion were seeded into 180 microplate wells. 24 of these, i.e. 13%, demonstrated significant production of the required monoclonal antibodies. In addition, monoclonal antibodies to factor IX had been produced as long ago as 1982 by A.H. Goodall et al, Blood 59(3), 664-670(1982), cited as reference AAH, by a similar method.

25. The new claims define the term "specific activity" more precisely. The clotting activity is determined by the method of D.E.G. Austen et al, "A Laboratory Manual of Blood Coagulation", Blackwell Scientific Publications Ltd. (1975), which is referred to in the specification at page 17 lines 16-19. The relevant pages are page 59 which describes the one-stage assay and pages 81-89 which describe how to make the determination graphically. (This description is for a two-stage assay, but the same principles of graphical evaluation apply to the one-stage assay). A copy of these pages is herewith made of record for further public information.

A claim corresponding to claim 2 is no longer present. The point involved was clarified at the interview.

26. The additional art made of record has been noted.

27. This paragraph apparently refers to the Austen et al reference. In view of the expanded definition of clotting activity given in the claims, it is believed that no further elaboration of the Austen et al reference should be required. A new page 17 expanded to include the "Method" and "Calculations" section of page 59 of the Austen et al reference could be provided if the Examiner wishes.

It is believed that all the requirements of the official action have been met and that all rejections should be found to be overcome.

Applicants have already filed two Information Disclosure Statements with Form PTO 1449 on this case. A signed copy of the second Form PTO 1449 was not returned with the official action. In case this IDS and PTO 1449 filed in February, 1987 have gone astray, a copy is provided herewith, together with a copy of the single reference cited which is European Patent Application Publication No. 200421A (Zymogenetics). This reference has been re-labelled "AAL". Additionally, applicants file herewith a further Form PTO 1449 which lists references AAA to AAG cited herein and an additional reference AA. AA is U.S. Patent 4,770,999 (Kaufman et al) issued December 13, 1988 having a priority date later than the priority date of the present application. Reference AA discloses the production of a factor

IX protein of low biological activity by culturing a chinese hamster ovary cell line in a medium containing vitamin K. It is evident that the specific activity of this protein is less than 9%: see column 12. This U.S. patent 4,770,999 and the present application are potentially in conflict, in view of the possiblity that Kaufman et al might have a date of conception before the foreign priority date of the present application. However, it appears that the two inventions are distinct and that neither party is in a position to copy the claims of the other.

In the circumstances, it is believed that this application is now in a form suitable for immediate allowance, and early action to that effect is requested.

Respectfully submitted,

NIXON & VANDERHYE P.C.

By: _____
Leonard C. Mitchard
Reg. No. 29,009

LCM:mss
2200 Clarendon Boulevard
14th Floor
Arlington, Virginia 22201
703/875-0400
Attachments: Declarations of Brownlee, Tuddenham, Gitschier and Marked copy of Brownlee Declaration, PTO Form 1449 and listed references AA-AAK & AAM, and Second Information Information Disclosure Statement, PTO Form 1449 and attached listed reference AAL.